The Coagulation of Protein by Sunlight.

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It was first shown by Dreyer and Hanssen (1) in 1917 that ultra-violet light produced a change in protein solutions which appeared to be similar to coagulation by heat. They exposed various solutions in quartz chambers to the light of a Bang lamp with iron and silver electrodes. Vitellin was found most easily coagulated, while globulin, albumin and fibrinogen showed a decreasing sensitivity to ultra-violet rays in the order mentioned. These investigators also discovered that acids markedly increase the rate of precipitation. Soret (2) had shown in 1883 that there are absorption bands in the extreme ultra-violet region of the spectrum of various proteins, *e.g.*, casein, ovalbumin, mucin and globulin. Tyrosine likewise has this band in the ultra-violet and Soret attributed to this constituent of the protein molecule its power of absorbing ultra-violet rays. In this connection Harris and Hoyt (3) carried out some interesting experiments on the protective power of various substances for paramœcium cultures exposed to ultra-violet radiations. They found that gelatin peptone, amino-benzoic acid, cystine, leucine and especially tyrosine possessed the power of detoxicating ultra-violet rays when placed as a thin layer of aqueous solution over paramœcium cultures under a quartz-mercury lamp. The toxicity of the radiations for paramœcia or protoplasm in general can be understood in the light of the discovery of Dreyer and Hanssen coupled with that of Soret.

From a physico-chemical standpoint Bovie (4) has published a study of the coagulation of proteins by ultra-violet light. By exposing solutions of crystalline ovalbumin, both dialysed and containing electrolytes, to the light of a mercury-vapour lamp, he came to the conclusion that there were two reactions involved in the coagulation of ovalbumin by ultra-violet light. The first is a photochemical one with a low temperature coefficient,—denaturation; and the second is one with a higher temperature coefficient of two and is dependent upon the electrolytes present,—coagulation. While using solutions dialysed against tap water Bovie made the observation that the protein appeared to become sensitive to light of longer wave-length, for his control tubes in glass were slowly coagulated.

Finally, from a medical point of view Schanz (5) has carried out a few experiments on this phenomenon with egg-white, blood serum and pig lenses.

From qualitative experiments only he claims to have discovered that certain substances, such as glucose, alcohol, lactic acid, urea and acetone, sensitise the serum proteins, while he states that certain German mineral waters protect them. He uses these observations to explain certain pathological conditions, especially old-age cataract.

When working with solutions of serum albumin which had been several times recrystallised, I had noticed that a marked change was produced in the solution while in glass vessels, so that the protein was almost quantitatively precipitated by mere exposure to sunlight. This precipitation occurred both with material which had been prepared by the alcohol-ether method and that obtained by direct crystallisation from serum, as I have described in a previous communication (6). A heavy precipitate appeared inside of 2 hours which proved soluble only in dilute alkali. The phenomenon seemed of such importance that a more thorough examination of the nature of the chemical reaction underlying it was undertaken.

As I have been unable to find any record of such a marked change in protein solutions by the action of sunlight in the literature, and further as the reaction was obtained with serum albumin of a purity such as has never, I venture to think, been achieved before, I attributed my observation of light instability to the purity of the preparations.

The following pages of experimental work are designed to show that the coagulant action of ultra-violet light on albumin in aqueous solution is also brought about by the visible rays of the spectrum when highly purified material is used. Experiments are described which show further that the nature of the reaction is identical with coagulation by heat in all points tested experimentally. That there are two reactions involved is demonstrated and changes in some physical constants of the pure protein solutions by the action of visible light rays are described as evidence towards an explanation of the primary reaction of protein coagulation.

METHODS.

Dialysis.—For the complete and rapid removal of contaminating electrolytes from solutions of albumin, the dialysing apparatus devised by Sörensen (7) has been used in a slightly modified form. It was necessary that the apparatus should possess the particular advantage of allowing the operator to maintain the solutions at their original concentration or to further concentrate them during dialysis.

The Preparation of Collodion Membranes.—Medicinal cotton wool was purified by extraction with boiling dilute alkali, followed by repeated changes of boiling water. The cotton was then dried and further extracted in refluxing absolute alcohol. After drying nitration was carried out as recommended by Blitz and von Vegesack (8). The washed and dried cellulose nitrate was dissolved in anhydrous alcohol-ether mixture (3 : 1) to form a 3 per cent. solution. This was applied by the Sörensen technique to a test-tube of ¾ inch diameter. Six or seven applications were given with a drying interval of 10 minutes between applications, and a final drying period of 5 to 7 hours before soaking in water.

The Dialysing Apparatus.—The general arrangement of the various parts was essentially that of Sörensen, although a smaller, slightly modified cell was used. The latter had a capacity of 250 c.c., while the membrane sack held 40 c.c. By the insertion of an extra limb in the upper part of the cell, very close to the neck, it was possible to remove the dialysate completely from the lower opening by the admission of air through the extra limb before introducing fresh water. This, I have found, allows of more rapid removal of the electrolytes present with a minimal use of distilled water, and also of the ready withdrawal of samples of the dialysate for analysis. The dialysis was carried out at room temperature, *i.e.*, 10–15° C., and the negative pressure found necessary varied from 5 to 20 cm. of mercury. The dialysate was replaced with distilled water twice daily. The only satisfactory way of determining whether the dialysing apparatus was in proper working order was to carry out a series of analyses on the successive dialysates withdrawn. Table I shows how efficiently the cell was functioning when it contained a solution of ovalbumin of 11·09 per cent. strength. After the fifth day no further positive test for the predominant impurity, $(NH_4)_2SO_4$, could be obtained. The solution contained 1·440 grm. $(NH_4)_2SO_4$ at the start and the first two days' dialysis served to remove more than 98 per cent. of the contaminating sulphate.

Table I.—Dialysis of Ovalbumin Solution, 11·09 per cent.

Day.	SO_4 test.	Protein test.	$(NH_4)_2SO_4$ content. Grm.	$(NH_4)_2SO_4$ content. Per cent. total.
1	+ + + +	—	1·2675	88·02
2	+ +	—	0·1473	10·23
3	+	—	0·0187	1·30
4	+	—	0·0023	0·16
5	—	—	0·0007	0·05
			1·4365	99·76

The $(NH_4)_2SO_4$ was determined by distillation with aeration as in a micro-Kjeldahl determination, using N/20 or N/100 H_2SO_4 in the distillate receiver and titrating excess acid by N/20 or N/100 $Na_2S_2O_3$ in the iodide-iodate titration.

The Preparation of Albumin.—Crystalline ovalbumin from one dozen egg whites was prepared by means of $(NH_4)_2SO_4$ and N/3 H_2SO_4, crystallising and recrystallising at the isoelectric point. Samples of the successive recrystallisations were dialysed in the apparatus previously described and the remainder after the third crystallisation treated likewise.

About 800 c.c. of horse serum were treated as described in a previous communication (6), using the direct method, and the crystals obtained recrystallised three times. This material was used for several of the experiments described below.

Technique of Light Exposure.—Small test tubes of ⅝ inch diameter of ordinary clear glass were used. About 5 c.c. of the fluid under examination were placed in a test tube or a small glass spectroscope box and exposed to sunlight or artificial light from which the infra-red rays had been removed by passage through a vessel with parallel sides containing clear water. A 5-inch lens with a focal length of 6 inches was used to concentrate the light rays, and it was placed about 5 inches distant from the light source in the case of the arc. The entire solution was exposed to the rays somewhat in advance of the focal point.

It was highly desirable to obtain a sufficiently powerful source of artificial light with some degree of constancy in place of the capricious sun. The light from a carbon arc, made by the Firma Carl Zeiss for use with their ultra-microscope and mounted on an optical bench, was used for several experiments. A current of 10–15 ampères was employed. This will bring about the same effect as sunlight if exposure be for a sufficient length of time. The intensity of the arc is only one-tenth that of mid-day sunlight, and thus an approximately similar result by artificial light requires about five times or more the length of exposure to sunlight. Another disadvantage in the use of the arc is the necessity of changing carbons frequently, for with the current strength used the carbons are completely burned up in 1½ hours, thus necessitating changing three or four times in the course of an experiment with ovalbumin.

Method of P_H *Determinations.*—The concentration of hydrogen ions in the various solutions used was determined colorimetrically, using the standard buffer mixtures devised by Clark and Lubs (9). The accuracy of the buffer mixtures was checked electrometrically by means of the potentiometer and the Barendrecht electrode. After some experimentation, the three following indicators were selected as most suitable for the purpose.

P_H.	Indicator.
3·1—4·4	Methyl orange.
4·4—6·0	Methyl red.
6·4—7·8	Neutral red.

To obviate possible errors due to the presence of proteins and salts, the method of dilution was adopted. Thus 1 c.c. of fluid was pipetted into a test tube, 4 c.c. of CO_2-free water added, and a suitable quantity of the indicator. A comparator was employed in cases of turbidity or foreign coloration. The procedure of dilution was tested and the values obtained by the use of the indicators were verified electrometrically on test solutions.

RESULTS.

Experiment 1.—*Susceptibility of different Crystallisations to Light Change.*

A series of test tubes were prepared containing solutions of the first three crystallisations of ovalbumin. These were approximately of 2 per cent. strength and had been dialysed free from electrolytes. They were adjusted to the same P_H by addition of a few drops of N/10 HCl. Another series was prepared containing 1 c.c. of the different ovalbumin solutions and 2 c.c. of a buffer mixture, made up of equal volumes of N/1 CH_3COOH and N/1 CH_3COONa, giving a P_H of 4·74. The results are shown in Table II.

Table II.—Susceptibility of Different Crystallisations to Illumination.

Test.	Mixture.	P_H.	Result.
1	3 c.c. solution, 1st crystallisation	5·4	—
	1 c.c. ” ” + 2 c.c. buffer...	4·6	—
2	3 c.c. ” 2nd ”	5·4	—
	1 c.c. ” ” + 2 c.c. buffer...	4·7	+
3	3 c.c. ” 3rd ”	5·4	—
	1 c.c. ” ” + 2 c.c. buffer...	4·7	+ +

Time of exposure to sunlight, 6 hours.
Albumin concentration of unbuffered solutions, 2 per cent.

From the results recorded in Table II, where precipitates appeared in the buffered solutions of the second and third crystallisations of ovalbumin, it became evident that repeated crystallisation tended to render the albumin more sensitive to light. It is to be noted that no precipitate appeared in the unbuffered solutions. Now, between the unbuffered and buffered solutions there existed three differences. The buffered solutions possessed a slightly lower concentration of protein, a slightly higher concentration of hydrogen ions, and a much greater electrolyte-content than the unbuffered solutions.

The precipitate obtained was soluble in alkali but insoluble in excess water or acid. The amount was small, and only a low fraction of the total protein in solution.

Experiment 2.—*The Influence of* P_H *Variation on the Light Reaction.*

To determine the possible effect of P_H variations, a series of tests were prepared containing ovalbumin solution of the third crystallisation product, to which a few drops of acetic acid of 10 per cent. strength had been added. A control tube containing the same albumin solution, with buffer mixture, was illuminated at the same time. Further controls of tests 1 and 5 were prepared, and kept at the same temperature in the dark. The proportions and results are recorded in Table III.

Table III.—Effect of P_H Variations on Unbuffered Ovalbumin Solutions.

Test.	Mixture.	P_H.	Result.
1	5 c.c. solution + 2 c.c. buffer solution	4·8	+
2	5 c.c. ,, + 0·05 c.c. acetic acid..................	4·7	—
3	5 c.c. ,, + 0·10 c.c. ,, ...	4·6	—
4	5 c.c. ,, + 0·15 c.c. ,, ...	4·45	—
5	5 c.c. ,, + 0·20 c.c. ,, ...	4·2	—

Time of exposure to sunlight, 8 hours.
Concentration of ovalbumin, approximately 2 per cent.

The P_H values were determined at the close of the experiment, and the results were only seen in reality when the tubes were all brought to the isoelectric point of ovalbumin. On neutralising the acidic solutions with dilute NaOH, a marked precipitate appeared, about P_H 5·0 (4·8–5·4), which was soluble in excess of alkali, but reprecipitated at the same P_H on adding acid. This precipitate remained undissolved on further acidification of the solution. The volume of the precipitate varied directly with the original degree of acidity. The two unilluminated control tubes showed no precipitate whatever when adjusted to a P_H of 5·0. This experiment shows that acid increases the rate of the light reaction, but that in solutions very low in their concentration of electrolytes, the albumin is not precipitated. This observation brings the reaction in very close similarity to heat coagulation. The absence of a precipitate in the unbuffered solutions of Experiment 1 can now be explained, in the light of the fact brought out by Experiment 2.

A wider range of P_H variations was next tried, with the purpose of discovering the influence of alkali on the reaction. Phosphate and phthalate buffer mixtures were employed, with a solution of ovalbumin three times recrystallised. The time of exposure to very bright sunlight was 5 hours. P_H determinations were made both before and after the exposure. The results are recorded in Table IV. Each test consisted of 2 c.c. of buffer and 1 c.c. of ovalbumin solution. The concentration of ovalbumin in the original solution was 2 per cent., and in the illuminated tubes was thus about 0·7 per cent. Controls of tests 1 and 6 were prepared and kept in the dark, with negative results.

Table IV.—Effect of P_H Variations on Buffered Ovalbumin Solutions.

Test.	P_H.		Result after exposure.	At P_H 4·8.
	Before.	After.		
1	3·0	3·5	+ + + +	+ + + +
2	4·0	4·3	+ +	+ +
3	4·8	5·1	+	+
4	5·4	5·6	opalescent	+
5	6·0	5·6	,,	+ +
6	7·6	6·5	,,	+ + + +

Time of exposure to sunlight, 5 hours.
Concentration of ovalbumin, 0·7 per cent.

At the conclusion of the period of illumination, the tubes on the acid side of P_H 5·4 showed precipitates, the degree of which varied directly with the hydrion concentration. The tubes on the alkaline side of P_H 5·4 were merely opalescent. On acidulation to a P_H of 4·8, however, there appeared precipitates, the degree of which varied directly with the hydroxyl ion concentration of the original solution. The comparison between heat coagulation and light coagulation is thus brought even closer. Both acids and alkalies increase the rate of the light reaction, and during this change there is a removal of H or OH ions from the solution depending upon its reaction.

Experiment 3.—*What Physical Changes does the Protein undergo before Flocculation?*

In order to discover whether some physical change in the solution which was being subjected to light bombardment might be used as an indication of the rate of the light reaction, several properties were investigated. It was my intention to study the first reaction quite separately, if possible, from the second, involving the flocculation of the altered protein.

(A) *Optical Rotation.*—A 100-mm. tube, filled with the dialysed solution of the third crystalline product of ovalbumin, was exposed to the light of the arc, and readings of the optical rotation were made frequently by removing the tube to a Hilger polarimeter, with direct-vision spectroscope, and observing the rotation (α_E) of the green line of a quartz mercury-vapour lamp ($\lambda = 546{\cdot}1\ \mu\mu$). The instrument was accurate to 0·01°. The observations were as follows:—

Table V.—Effect of Illumination on Optical Rotation.

Time of illumination.	α_E.	$[\alpha]_E$.
minutes.	°	°
0	0·79	−36·60
10	0·81	−37·60
30	0·84	−38·99
45	0·87	−40·38
105	0·87	−40·38

Concentration of ovalbumin, 2·16 per cent.
α_E = observed rotation.
$[\alpha]_E$ = specific rotation.

The results in Table V show that one of the effects of radiations of the visible spectrum on pure crystalline ovalbumin is an increased power of optical rotation. This increase in specific rotation was observed in a solution that was entirely free from precipitate. A control tube of the same length, and containing the same solution, was read consecutively with the illuminated tube. It gave the same reading of 0·79 throughout the experiment.

An experiment to confirm the above observation was tried, using crystalline serum albumin which had previously been exposed to sunlight for 2 hours. The protein thus exposed was completely coagulated, for the solution contained $(NH_4)_2SO_4$ to the extent of 1 per cent. It is to be noted that in this experiment the serum albumin of 3·46 per cent. strength was completely coagulated inside of 2 hours, whereas ovalbumin in similar concentration is only partially coagulated after 6 to 8 hours' exposure. Now, the original unilluminated serum albumin had had a specific rotation of −78·60° by the green line of the mercury spectrum ($\lambda = 546{\cdot}1\ \mu\mu$). To the coagulum, with its supernatant liquid of about 100 c.c., were added fifteen drops of concentrated ammonia, and complete solution was thus brought about. This solution was examined in the polarimeter, and the specific rotation of the serum albumin was found to have risen to almost 5° above that of the undenatured substance. Now, in the case of unilluminated ovalbumin, I have been able to show that the specific rotation is a constant only at the isoelectric point (6). If acid be added, the value for [α] is increased. If alkali be added, the value is temporarily decreased. In order to discover whether this was also a fact with the denatured albumin, two or three drops of HCl (20 per cent.) were added, so as to adjust the P_H of the solution near to the isoelectric point. The specific rotation rose almost 5° to a constant value of −87·92° in several hours. The solution was again made alkaline by means of a few drops of ammonia and again the value for the specific rotation fell to −83·0°. The same slow rise to the higher value was observed as in the case of unilluminated ovalbumin. It is to be noted that the fluctuations from −83·0° to −89·6° are around a much higher mean value than the one for undenatured material, −78·6°. The significance of this observation is discussed in a later section. Table VI gives the data in detail.

Table VI.—Optical Rotation of Denatured Serum Albumin.

Time interval.	Solution.	Temperature.	P_H.	α_E.	$[\alpha]_E$.
		°		°	°
—	Original	15	5·6	−2·71	−78·6
—	Denatured + NH_3	14·2	7·4	−2·88	−83·3
1 hour	„ + HCl	15	5·4	−3·01	−87·1
5 hours	„	15	5·4	−3·04	−87·9
12 hours	„	14·5	5·4	−3·04	−87·9
5 mins.	„ + NH_3	15	7·3	−2·87	−83·0
2 days	„	15	7·3	−2·95	−85·3
3 days	„	14·5	7·3	−3·09	−89·4
1 day	„ + NH_3	14·5	7·6	−3·10	−89·6
1 day	„	14·2	7·6	−3·09	−89·4

Concentration of serum albumin, 3·46 per cent.
Time of previous exposure to sunlight, 2 hours.

(B) *Viscosity and Surface Tension.*—A solution of ovalbumin, three times recrystallised and dialysed free from sulphate, was used in the following experiments. Changes in viscosity were measured by an Ostwald viscosimeter immersed in a thermostat kept at 20° C. and regulated so that the temperature was constant to 0·1°; 5 c.c. were used for a determination, measured by means of a calibrated pipette.

The surface tension was measured by means of a Traube stalagmometer (Gerhardt No. III), standardised by distilled water and found to give 37·10 drops. The instrument was accurate to 0·05 of a drop. It should be pointed out here that in such a measurement the tension measured is between air and the dispersion medium, not between dispersion medium and disperse phase. Any change the latter might undergo, however, would very probably be shown in the former, though not of necessity.

The results obtained as shown in Table VII indicate very small changes. It is well to point out that the amount of albumin undergoing change is very small relative to the amount present. This I have repeatedly shown by heat coagulation of the remaining solution at its isoelectric point. The arc light was used in this experiment and the solutions exposed in glass spectroscope boxes.

Table VII.—Effect of Illumination on Viscosity and Surface Tension.

Exposure time.	Viscosity.	$\eta \times 10^3$.	Surface tension.	γ.
hours.	seconds.		drops.	
0	19·80	11·75	39·00	69·74
1·5	20·60	12·23	39·10	69·56
3·0	20·80	12·34	39·60	68·70
4·5	21·00	12·47	39·70	68·52

Concentration of ovalbumin, 2·10 per cent.

There is thus a slight decrease in surface tension (γ) and a slight increase in viscosity (η), but the changes are so slight that as a means of following the rate of reaction they are unsuitable. It is, however, significant that such changes occur, indicating as they do underlying chemical changes while the denatured particles remain dispersed. The work is being repeated with serum albumin solutions.

Experiment 4.—*The Effect of various Substances on the Rate of Denaturation.*

For the following tests a solution of serum albumin, twice recrystallised, was used. It still contained the mother liquor adherent to the crystals, so that there was an appreciable quantity of $(NH_4)_2SO_4$ present (about 1 per cent.). This solution was quickly affected by sunlight, depositing a massive precipitate in an hour if the sunlight were intense. It was denatured by the arc light on 2 hours' exposure. The precipitate was found to be soluble in excess water, acid or alkali. The solution in water or acid could only be brought about with precipitates formed quickly and freshly deposited. This observation is comparable with that of Michaelis and Rona (10) on heat coagulation of serum albumin. If the precipitate forms slowly, its solubility in both acid and water is lost. I am not able to say whether the resolution is an indication of a truly reversible reaction, or whether it merely indicates on the part of the colloid the ability to reassume a charge and disperse itself, as is the case of pure gelatin, about its isoelectric point. Michaelis and Rona state that in the case of heat coagulation it is a true reversion, but adduce no evidence for their belief. In this connection it is interesting to recall the observation of Corin and Ansiaux (11) that if freshly coagulated egg albumin be vigorously shaken it is again dispersed and a clear solution results. This point is discussed under the theory of the whole phenomenon.

The effect of adding various substances was tried on the serum albumin solution, and control tubes were prepared in each case and kept in the dark at the same temperature. Electrolytes in moderate concentration were found to increase the rate of denaturation, *e.g.*, NaCl, $(NH_4)_2SO_4$, KSCN. Traces of alcohol, acetone and toluene did likewise. Glucose and ether had no apparent effect. To be in a position to study the effect of various substances, however, on the rate of coagulation, a quantitative method would have to be devised. From the above experiments it would appear as if any substances with dehydrating power would increase the rate of denaturation. I am at present working upon this subject.

The Nature of the Light Reaction.

From the foregoing experiments it will be apparent that the reaction brought about by sunlight is very similar to heat coagulation, if not identical with it. The main facts associated with the phenomenon of heat coagulation may be briefly summarised for purposes of comparison. Chick and Martin (12) have conclusively demonstrated that, under certain circumstances, *i.e.*, when the water present is in large excess and the hydrogen ion concentration is kept constant, the reaction can be proved to be of the monomolecular order. The reaction is between protein and water and the effect of temperature is merely to accelerate it. It has an extraordinarily high temperature coefficient and the velocity is influenced by a number of conditions, especially acid and alkali. During coagulation, if the reaction be on the acid side of the iso-electric point, hydrogen ions are removed from solution; if the reaction be on the alkaline side hydroxyl ions are removed. The degree of removal of the H or OH ions is dependent on the total concentration of acid or alkali and the P_H of the solution. Heat coagulation of albumin consists of two processes: (1) a reaction between protein and water (denaturation); (2) the separation of the altered protein in a particulate form (agglutination). From the investigation of Sörensen and Jürgensen (13) on ovalbumin, and of Michaelis and co-workers (14) on serum albumin, the maximum flocculation of denatured protein has been demonstrated to occur only at its isoelectric point. It is interesting to note here that Michaelis and Davidsohn (15) have found that the isoelectric point of denatured serum albumin (P_H 5·4) is not the same as natural serum albumin (P_H 4·6). Hardy (16) recognised the double nature of heat coagulation of egg white as long ago as 1899 and considered the primary change as one of an emulsoid colloid to a suspensoid type, which was readily precipitated by small quantities of electrolytes. Michaelis endorsed this view from his work on serum albumin. Most workers have confined themselves to a study of the conditions governing flocculation, and the more fundamental primary change from a natural protein to one which is readily precipitated on neutralisation of the charge carried by the particles has been neglected.

The explanation of coagulation generally accepted is due to Hofmeister and Pauli. They conceive of the primary change as one of dehydration involving the internal neutralisation through the loss of the elements of water of terminal NH_2 and COOH groupings. From a study of the coagulation of casein by alcohol Robertson comes to the same conclusion.

Now in the change brought about by sunlight there are two reactions. The first change involves a simple chemical one, for which the light is responsible, which causes an increase in optical rotatory power and in viscosity, a decrease in surface tension and a decrease in H or OH ions depending on the reaction of the medium. The second stage will only precede if the solution is at or near the isoelectric point. It is materially aided by small amounts of electrolytes or dehydrating agents such as alcohol or acetone. Under certain circumstances the second stage is reversible. The explanation would seem to lie in the ability of the albumin to reassume charged ions so long as agglutination has not proceeded too far. In other words the forces of repulsion of similarly charged ions attached to the colloidal particles are sufficient to overcome the adhesive forces of the albumin flocculi. It is conceivable that in the primary stage not only do we have internal anhydride formation within single colloidal aggregates, but that as coagulation proceeds this anhydride formation extends to linkages between two or more colloidal aggregates. The determination of H or OH ions reveals a diminution during coagulation. This can be readily explained on the basis of the amphoteric nature of the protein. On the acid side of the isoelectric point the protein is an acid and must possess some free COOH groupings. If these become neutralised, as in anhydride formation, the acidity of the solution will be diminished. The converse will hold true in alkaline solution where NH_2 groups function as basic influences.

The explanation of the primary reaction as simply involving an internal dehydration seems most reasonable on account of the great number of ways in which denaturation can be brought about. Light, heat, mechanical shock, undue strain as in the surface of expanding air bubbles, or any marked double phase such as is produced by the mixing of two immiscible liquids, acidity, alcohol, all these agents bring about a change such that the protein becomes insoluble. The action of alcohol is interesting in that in this reagent we possess one which can bring about both changes, but that if conditions are regulated only one will take place. Thus it is possible to remove the water of solution or solvate water from the colloidal particles by means of 95 per cent. alcohol in the cold. Flocculation is thus brought about, as is the case by concentrated salt solutions, which is reversible and not accompanied by the change called denaturation. If, however, alcohol be added to a protein solution in like manner, but at 30° C., then the power of alcohol as a dehydrating agent appears so augmented that not only does it remove solvate water, but also may be pictured as inducing internal anhydride formation (denaturation). The precipitate thrown down is irreversible. In the case of mechanical coagulation, the phenomenon is much more readily produced if a dehydrating agent is present in quantity. It would be interesting to know if it would occur at all should every trace of electrolyte be removed from the solution.

From the above discussion, it would appear that the *rôle* of light as a coagulating or denaturating agent is similar to that of heat—a catalyst of the primary fundamental chemical reaction. That certain substances can aid or hinder its action is very probable from preliminary observations. Certain it is, from the results of Experiments 3 and 4, that serum albumin is many times more sensitive to light than ovalbumin. In this connection, it is interesting to note that the purest serum albumin solutions still contained a minute amount of pigment. I am unable to say whether the greater sensitivity of serum albumin was or was not due to its influence.

SUMMARY.

Serum albumin and ovalbumin which have been several times recrystallised become sensitive to intense light, either sunlight or strong arc illumination, from which the infra-red and ultra-violet rays have been removed. Serum albumin is many times more easily affected than ovalbumin.

The change brought about by light has many of the characteristics of heat coagulation. It consists of two separate reactions: (1) denaturation—a primary chemical change; (2) flocculation—the precipitation of denatured particles.

The primary reaction is accompanied by increase of viscosity and optical rotatory power, and decrease of surface tension. The velocity of the primary change is increased both by acids and by alkalies. During the reaction, H ions are removed if the P_H be on the acid side of the isoelectric point; if on the alkaline side, OH ions are removed.

The secondary reaction does not follow if the solution be free from electrolytes. It is brought about by adjusting the solution to about the isoelectric point of the albumin, P_H 4·8–5·4. Under conditions when the precipitate is formed readily, it will go back into solution on the addition of

acid or excess water. If the precipitate is formed slowly, it is only dispersed by alkali.

Certain substances act as accelerators for the reaction, such as alcohol, acetone, $(NH_4)_2SO_4$, NaCl, KSCN. The mechanism of the reaction is discussed.

In conclusion, I wish to take this opportunity of expressing my thanks to Prof. F. G. Hopkins for much kind criticism and encouragement.

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